97. The method of Claim 96 wherein said rigid porous vessel is a ceramic cube.

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98. The method of Claim 90 wherein said simple sugar is glucose.

99. The method of Claim 98 wherein said glucose is present in said serum-free medium in an amount of about 4.5 grams per liter.

REMARKS

Claims 80-99 have been added in order to define further embodiments of the invention.

Claims 60-79 stand rejected under 35 U.S.C. 102(e) as being anticipated by Johnstone, et al., as evidenced by www.voightglobal.com/Cellgro_basal_liquid.htm (also hereinafter referred to as the "Cellgro web page"). This rejection is respectfully traversed.

The present invention, in one aspect, is directed to a process for producing chondrocytes, as defined broadly in Claim 60, and to a process for inducing chondrogenesis in mesenchymal stem cells, as defined broadly in Claim 70. Such processes are effected by culturing mesenchymal stem cells in a chemically defined serum-free medium in vitro wherein the mesenchymal stem cells are associated in a three-dimensional format. The chemically defined serum-free medium comprises (1) a chemically defined minimum essential medium; (2) ascorbate or an analog thereof; (3) an iron source; (4) insulin or an insulin-like growth factor; (5) at least one chondroinductive agent or

factor; and (6) a simple sugar, wherein the simple sugar is present in the medium in an amount of from about 3g/l to about 7g/l.

Applicants have discovered, as shown in the examples, that by culturing mesenchymal stem cells in a chondrogenic medium which includes a simple sugar at a concentration of from about 3g/l to about 7g/l, one obtains improved differentiation of mesenchymal stem cells into chondrocytes, as opposed to media which have a lower sugar concentration, such as, for example, media which have a glucose concentration which is the standard concentration present in "low glucose DMEM" (1g/l).

In another aspect of the present invention, as defined broadly in Claims 80 and 90, the mesenchymal stem cells are cultured in a chemically defined serum-free medium as hereinabove described, wherein the at least one chondroinductive agent or factor comprises TGF-β3.

Applicants also have discovered that TGF-β3 is a more effective chondroinductive agent than those used previously, such as dexamethasone, BMP-2, BMP-4, TGF-β1, inhibin A, chondrogenic stimulating activity factor, collagen Type I, or retinoic acid. For example, in Example 3, TGF-β3 was found to have an improved effect on chondrogenic differentiation of human mesenchymal stem cells *in vitro* when compared to TGF-β1.

As stated previously in Applicants' Amendment filed August 15, 2002, Johnstone does not disclose or even remotely suggest to one of ordinary skill in the art that the chondrogenic medium may include a simple sugar in an amount of from about 3g/l to about 7g/l, and that the only specific example of medium used by

Johnstone, i.e., a medium which includes Dulbecco's Modified Eagle's Medium-Low Glucose (DMEM-LG), has a glucose concentration of 1 g/l.

The Examiner has taken the position that Johnstone anticipates the present invention because Johnstone states that DMEM may be employed in a chondrogenic medium, and (i) the Cellgro web page teaches that DMEM contains 1 to 4.5 g/l of glucose, and (ii) the Sigma web page teaches that the original DMEM formula contains 1 g/l of glucose and that 4.5 g/l of glucose has proven to be optimal in cultivating certain cell types.

In response, Applicants assert that the only specific concentration of glucose disclosed by Johnstone is 1 g/l. Johnstone provides no suggestion to one of ordinary skill in the art that the chondrogenic medium can have a simple sugar concentration from 3 g/l to 7 g/l.

The Cellgro web page adds nothing to Johnstone. All that the Cellgro web page discloses are various formulations of DMEM, one of which contains 1 g/l of glucose, and others which contain 4.5 g/l of glucose. The Cellgro web page does not provide any suggestion to one of ordinary skill in the art as to the types of cells which may be cultured in DMEM including 4.5 g/l of glucose, or that DMEM including 4.5 g/l of glucose may be employed in a medium for culturing mesenchymal stem cells in order to enable the mesenchymal stem cells to differentiate into chondrocytes.

All that is stated in the Sigma web page is that an "alteration" of Dulbecco's Modified Eagle's Medium with 4.5 g/l

of glucose has proven to be optimal in cultivating certain cell types. Nothing in Sigma, however, states which specific cell types may be cultivated in DMEM containing 4.5 g/l of glucose, and there is not even the remotest suggestion in Sigma to one of ordinary skill in the art that one can use DMEM with 4.5 g/l of glucose as part of a medium for culturing mesenchymal stem cells, whereby the mesenchymal stem cells will differentiate into chondrocytes.

Assuming solely for the sake of argument that it was known to provide a culture medium that contained glucose in an amount of 4.5 g/l, neither Johnstone nor the Cellgro and Sigma web pages disclose or even remotely suggest to one of ordinary skill in the art that such a medium, or any culture medium having a simple sugar concentration from 3 g/l to 7 g/l may be used as part of a culture medium for mesenchymal stem cells for enabling the mesenchymal stem cells to differentiate into chondrocytes. The only specific teaching in the combination of Johnstone Cellgro, and Sigma with respect to the concentration of glucose in a chondrogenic medium for culturing mesenchymal stem cells is to use a concentration of 1 g/l of glucose in such a medium. Therefore, Johnstone teaches away from the present invention, and such teaching away from the invention is indicative of non-anticipation and non-obviousness. (See W.L. Gore & Associates, Inc. v. Garlock, Inc., 220 U.S.P.Q. 303 (C.A.F.C. 1983), at 312; United States v. Adams, 383 U.S. 39 (1966).)

In addition, Johnstone discloses as chondroinductive agents glucocorticoid such as dexamethasone, a bone morphogenic protein, such as BMP-2 or BMP-4, TGF-β1, inhibin A, or chondrogenic stimulating activity factor. The examples, i.e., Examples 1 through 3, employ dexamethasone, TGF-β1, and BMP-2,

respectively, as the chondroinductive agents. Johnstone does not disclose or even remotely suggest to one of ordinary skill in the art that TGF-β3 may be used as a chondroinductive agent, or that TGF-β3, when employed in a chondrogenic medium, provides for improved differentiation of mesenchymal stem cells into chondrocytes. Thus, Johnstone also does not negate the patentability of the embodiments of the claimed invention defined by newly added Claims 80-99.

For the above reasons and others, this application is in condition for allowance, and it is therefore respectfully requested that the rejections under 35 U.S.C. 102(e) be reconsidered and withdrawn and a favorable action is hereby solicited.

Respectfully submitted,

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